Baffling Butadiene

The United States was a nation at war in 1942, the demands of which included quickly establishing a number of industries aimed at supplying crucial material for ensuring military success overseas. Critical among these was the establishment of the synthetic rubber industry, which began in 1942 under federal sponsorship.

Just as it was in 1942, 1,3-butadiene, the gaseous hydrocarbon commonly referred to simply as butadiene, is an important major commodity product of the petrochemical industry. Butadiene has a pungent odor like gasoline that can be detected at about one to two parts per million (ppm). It is highly flammable and is handled as a liquified compressed gas. Its principal use is in the manufacture of butadiene–styrene copolymer elastomer, of which more than 60% is used for tires. The elastomer is also used to prepare a variety of other synthetic rubber products and chemicals such as adiponitrile, a nylon precursor, and is used in the manufacture of plastic food wrappings, sponges, hoses and piping, footwear, luggage, packaging, and a variety of molded products.

In the United States, almost 100% of the nearly 3 billion pounds of butadiene is produced as a co-product of ethylene manufacture. Most butadiene is extracted from a mixed butenes stream, or crude butadiene, which is a by-product of ethylene production. U.S. extraction facilities are located in Texas and Louisiana. These extraction facilities are typical petrochemical plants: treelike fractionating towers predominate a steely maze of pipelines and structures housing a variety of pumps, reboilers, heat exchangers, valves, and bunkerlike control buildings. These structures are open air, some covering up to 30 acres, but all hydrocarbons are fully contained to prevent fire and explosion.

Occupational exposures to butadiene can occur during production, storage, and transport of the monomer or its chemical mixtures. Decontamination and maintenance of processing equipment, sampling and analyzing quality control samples, and loading or unloading tank trucks or rail cars are typical operations during which exposure may occur. Equipment leaks account for 99.5% of the atmospheric emissions of butadiene from industry. Together with the much larger magnitude of butadiene emissions from vehicle exhaust, industrial emissions may account for localized "hot spots" within overall patterns of human exposure. At the perimeter of the industrial complex in Port Neches, Texas, where butadiene and styrene-butadiene rubber has been produced since the early 1940s, levels as high as 2 ppm were detected in the air as recently as 1990.

An estimated 3100 workers are exposed to butadiene in 11 U.S. production facilities, which include crude, monomer, and terminal facilities. Monomer plants are highly automated and involve fewer workers than the other facilities. In addition to these are an estimated 4200 workers at 34 butadiene-consuming plants nationwide who are also potentially directly exposed to the monomer. Data compiled from a 1990 National Occupational Exposure Survey by the National Institute of Occupational Safety and Health suggest that approximately 50,000 workers in end-user industries are potentially exposed to butadiene through contact with a variety of butadiene subproducts.

Controversy surrounding health effects of occupational inhalation exposure to butadiene is focused largely on its potential as a human carcinogen. At the time of industry start-up during World War II, human carcinogenicity associated with butadiene exposure was unknown. Since January 1976, when two former workers at adjacent synthetic rubber production facilities in Texas died of leukemia, dozens of scientific reports and commentaries regarding butadiene carcinogenicity have appeared in the literature, the authors at times crossing verbal broadswords with an almost audible clank.

UPI/Bettmann

Setting the wheels rolling. Senator Raymond Willis, of Indiana, left, and "Rubber Czar" William M. Jeffers unveil the first all-synthetic tire in 1942.

Caught in the middle of this controversy is the U.S. Occupational Safety and Health Administration (OSHA), which must weigh all evidence before deciding on any reduction in the workplace permissible exposure limit (PEL) for butadiene. OSHA is committed to reducing worker exposure to the hydrocarbon. The current 8-hour, time-weighted average workroom PEL for butadiene is 1000 ppm, a figure set in 1981 aimed at preventing irritation to the eyes and upper respiratory tract.

Today, because butadiene has been shown to cause tumors in laboratory mice and rats and because evidence suggests it is carcinogenic in humans exposed occupationally, EPA and the International Agency for Research on Cancer rank butadiene as a probable human carcinogen. In response to those same research findings, OSHA has proposed lowering the PEL to 2 ppm, but impelled by laboratory findings of low-dose exposure effects in mice, the agency later this year may set an even lower standard. The American Conference of Governmental Industrial Hygienists (ACGIH) last May listed butadiene on its Notice of Intended Change. In May 1994, an ACGIH subcommittee will review this notice, which calls for lowering the workplace threshold limit value (TLV) from 10 ppm to 2 ppm. The ACGIH designation of butadiene is A2: suspected carcinogen. "Butadiene is definitely the bad actor in butadiene/styrene co-exposures," says Calvin Wilhite of the ACGIH TLV committee.

"A range of concerns have been expressed on butadiene's potential for human carcinogenicity," says Ronald Melnick, NIEHS senior toxicologist. "It includes the very concerned to those who claim it doesn't exist for humans."

The contention over this issue is revealed in editorial commentary and scientific reports since 1989 that demonstrate the authors' passions toward findings and implications. Indeed, time and again, what some emphasize to drive home a point, others view as misinterpretations of data. And when these same scientists convene at symposia to shed light on their latest work, it is not without some heat. And yet summaries of joint conferences read like exercises in restraint and editorial balance; they betray no trace of the wrangling that surely must have accompanied reaching agreement on final wording.

The Model and the Message

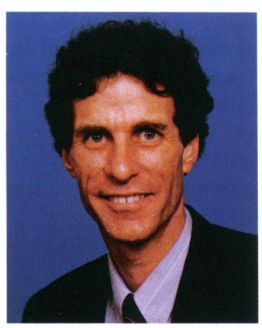
Where do scientists agree on butadiene? None deny its potency as a carcinogen in mice. The carcinogenicity of inhaled butadiene has been studied in Sprague-Dawley rats by the International Institute of Synthetic Rubber Producers and in B63CF$_1$ mice by researchers in the U.S. National Toxicology Program (NTP). A 1988 NIEHS international symposium on butadiene published in *EHP* (volume 86) in 1990 included reports of butadiene-induced neoplasms at multiple organ sites in rats (pancreas, uterus, Zymbal gland, mammary gland, thyroid, testis) and mice (lymphomas and neoplasms of the heart, forestomach, lung, liver, mammary gland, ovary, and preputial gland). In those studies,

rats were exposed to butadiene by inhalation at 1000 and 8000 ppm, and mice were exposed at 625 and 1250 ppm.

Since then, an expanded NTP study on male and female mice exposed to butadiene inhalation at 0, 6.25, 20, 62.5, 200, and 625 ppm for up to 2 years has been completed. Melnick and his colleagues observed butadiene carcinogenicity in mice at all exposure levels, including early, extensive induction of malignant lymphomas, which again was the major cause of early death for both sexes exposed to 625 ppm. Incidence of lymphomas also increased for females at 200 ppm. Induction of uncommon hemangiosarcomas of the heart were observed at concentrations as low as 20 ppm. Malignant lung tumors occurred at the 6.25 ppm level, the lowest concentration ever used in a long-term cancer study of butadiene. In Melnick's stop-exposure studies of butadiene in mice, carcinogenesis in multiple organs was induced after only 13 weeks of exposure, and for lymphoma the concentration of butadiene was a greater contributing factor than duration of exposure.

Thus, butadiene exerts a clear carcinogenic effect after relatively short periods of exposure and at multiple organ sites in mice exposed by inhalation in long-term studies. In rats, the effect is less pronounced and requires much larger doses to induce cancer and target organs differ with the exception of mammary gland.

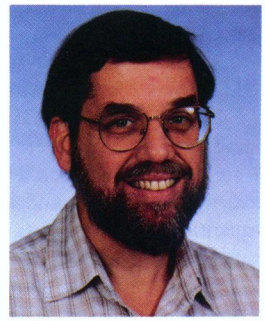
According to Melnick and NIEHS coauthor James Huff, the finding most relevant to human risk is that mice show "good correspondence" with reported associations between occupational exposure to butadiene and excess mortality from lymphatic and hematopoietic cancers. This finding strengthens the argument that mice, rather than rats, are a better experimental surrogate for humans. Furthermore, Melnick and Huff suggest that perhaps the rat is uniquely insensitive to leukemia/lymphoma induction. Similar to butadiene, the human leukemogen benzene produces lymphomas in mice but not in rats. Rats are also much less sensitive to radiation-induced leukemia than mice. Melnick and Huff conclude that, based on the cumulative weight of evidence, there is a causal association between exposure to butadiene and human cancer, and there is a vital public health need to reduce exposure to this chemical.



Bill Touchberry

James Bond—Research in rats and mice needs to move from predicting to studying what is actually occurring.

Other reports, however, have questioned the appropriateness of extrapolating data on the carcinogenicity of butadiene in mice to carcinogenicity in humans. Arguments include laboratory observations of species differences in butadiene metabolism. *In vivo* studies have illustrated species differences among mice, rats, and monkeys in low-level butadiene absorption, metabolism, and retention. For example, when looking at concentrations of the metabolite butadiene monoepoxide in the blood of all three species after inhalation of butadiene concentrations ranging from 8 to 8000 ppm, monkeys always had lower blood epoxide concentrations than rats or mice. Metabolism of butadiene by rodents, in general, may be qualitatively different from primates. Recent studies of butadiene metabolites in people have also pointed to these rodent-primate differences based on metabolites in urine. This finding points to something toxicologists have always known: rodents are not perfect surrogates for humans.



NIEHS

Ronald Melnick—There is a causal relationship between exposure to butadiene and human cancer.

Another focus, such as work underway at the Chemical Industry Institute of Toxicology (CIIT), has been on specific butadiene epoxides in liver and lung tissue samples of rats, mice, and humans. Metabolic activation of butadiene involves oxidation mediated by various forms of cytochrome P450 (primarily P450 261) to DNA-reactive (genotoxic) metabolites such as butadiene monoepoxide. Significant species differences in *in vitro* rates of activation and deactivation of butadiene epoxides presumably support the idea that mice are far more sensitive to the carcinogenic activity of butadiene than rats or humans. Such interspecies comparisons of chemical metabolism lead some to conclude that human cancer risks from butadiene exposure are similar to that of rats, which are more resistant than mice to butadiene's carcinogenic effects. Findings obtained from this biochemically based model of butadiene pharmacokinetics are meant to flash a caution light in the eyes of regulators who rely on mouse studies to assess risks.

James Bond, head of biochemical toxicology at CIIT, cautions that metabolism does not entirely explain species differences in potency and organ-site specificity of butadiene carcinogenicity. "But I do think it's a critical factor," he says. "How those epoxides translate to a mutational event is unclear. Probably a combination of differences in metabolism, potential mutagenicity, and other species-related factors we don't understand may someday tell us why mice, for example, are more sensitive than rats."

Indeed, differences in carcinogenic sensitivity of rats and mice to inhaled butadiene are so far only suggestive of a toxicokinetic contribution. In a recent report, tissue concentrations of butadiene monoepoxide, predicted in rats and mice from a physiologically based pharmacokinetic model of uptake, tissue distribution, and metabolism of butadiene, could not account for differences in tumor incidence in these species.

Melnick has pointed out problems in data analysis of the earlier biochemically based modeling study which, when corrected, indicates humans are not necessarily as insensitive as had been thought. "Other factors crucial for carcinogenesis induced by butadiene may include accumulation of another epoxide, diepoxybutane, formation of DNA adducts, and efficiency of DNA repair." Still, none of these factors has been predicted by current physiologically based models.

Predictions by Melnick and colleague Michael Kohn's physiologically based pharmacokinetic model indicate release of butadiene from storage in fat after cessation of exposure would result in continued epoxide production in humans. This is not the case in mice, Melnick and Kohn point out, adding that results from acute exposure studies in animals may underestimate the risk to human health of repeated exposure to butadiene. Bond notes, however, that other investigators, including those at CIIT, also using physiologically based pharmacokinetic models, have come to different conclusions regarding the potential for storage of butadiene in tissues like fat. The major reason for the differences is the values for butadiene solubility in tissues that are used in the various models.

"We need to go beyond tissue dosimetry to understand the cancer process," Melnick says. "Differences between rats and mice in the doses of epoxide occurring internally are too small to explain differences in the carcinogenic effects in these species." Yet Bond points out that research needs to move away from simply predicting what might be occurring in rats and mice to actual studies of epoxide levels in blood and target tissues of these animals after butadiene exposures. "These are key studies for validation of the dosimetry models," he says.

In addition to the debate on metabolic differences are disagreements about retroviral background. Studies on the potential of endogenous murine leukemia virus (MLV) to influence susceptibility to butadiene-induced leukemia have also been cited as a reason for caution against extrapolating findings in mice to humans.

Studies by Richard D. Irons, director of molecular toxicology and environmental health sciences at the University of Colorado, determined that $B6C3F_1$ mice, which have

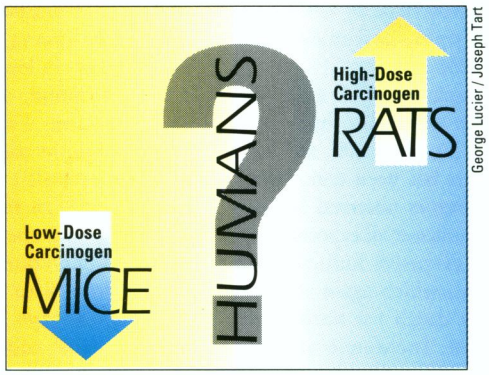
MLV, have a remarkably high incidence of butadiene-induced leukemia or thymic lymphoma. The incidence of lymphoma was fourfold (57% versus 14%) that of NIH Swiss mice that do not have the virus in a 1-year study of exposures at 1250 ppm butadiene. Irons points out that these findings are bolstered by the fact that target organ toxicity in the two strains is qualitatively and quantitatively identical after butadiene exposure during the preleukemic phase of the study. Says Irons, "Retroviral background influences the ultimate incidence but doesn't account for causation in toto."

According to other reports, however, butadiene is carcinogenic to Swiss mice, a strain without MLV and with a near-zero background rate for thymic lymphoma, and to Sprague-Dawley rats, in spite of metabolic and pharmacokinetic differences. Melnick and Irons agree that viruses alone cannot account for the carcinogenic effects of butadiene because the monomer induced a significant incidence of lymphoma in NIH Swiss mice. Moreover, Melnick adds, other genetic differences between the strains of mice could contribute to the different tumor rates. And because the NTP studies were only 52 weeks long, they do not necessarily reveal the full response for lymphoma induction by butadiene.

Mice exposed chronically to butadiene, treated with radiation, or bearing "white-spotted" or "steel" mutations show an identical pattern of disease, which includes a high incidence of leukemia and thymic lymphomas. This pattern may indicate a functional defect in a subpopulation of primitive hematopoietic stem and progenitor cells, Irons explains. These mutant mice lack the same subpopulations of primitive hematopoietic stem and progenitor cells that are susceptible to butadiene suppression in intact mice. This, he says, draws into serious question any reference to the mouse as a quantitiative model in risk assessment for humans. "If you use the mouse as a quantitative model, you do so at your peril," Irons warns.

Butadiene Epidemiology

In terms of occupational butadiene exposure, industrial hygiene data collected before 1980 are considered not particularly good because most exposures were well below the OSHA limit, few samples were used, and sampling methodology was not rigorous. More recent data compiled by NIOSH show the highest butadiene exposures occur in the monomer industry, where a mean airborne level of 5.9 ppm was found, with 7% of samples showing levels greater than 10 ppm, and 20% greater than 2 ppm, which is the level proposed by OSHA. In the polymer industry (including styrene-butadiene rubber, polybutadiene rubber, and other polymers), mean airborne exposure was 1.1 ppm, with 3% of samples showing more than 10 ppm, and 11% more than 2 ppm. However, maximum 8-hour time-weighted average exposures were frequently between 10 and 150 ppm, and in one case as high as 374 ppm, for jobs involving butadiene transfer, sampling, and maintenance. In end-user industries such as rubber tire plants and industrial hose plants, more than 100 samples contained no detectable levels of butadiene.



George Lucier / Joseph Tart

How much butadiene? While low doses produce tumors in mice and high doses are required for rats; it is not known where humans fall in the range.

Most epidemiological studies of exposure to butadiene have used a retrospective cohort design and included assessments based on mortality data. These studies have consistently indicated increased frequencies of lymphatic and hematopoietic cancers. Quantitative exposure data are lacking. A number of observations, however, appear compelling in terms of their applicability to assessing cancer risk in humans due to butadiene exposure.

Cohort mortality studies have shown lower overall mortality rates and lower mortality from all cancers in workers occupationally exposed to butadiene than rates for the general population. This may be evidence of a "healthy worker effect," in which study results may be skewed by the fact that someone who is working is more likely to be healthy. However, consistent among at least five epidemiological studies of workers exposed to butadiene is the finding of excess mortality from lymphatic and hematopoietic cancers. Moreover, excess mortality from these cancers among subgroups of workers, including black production workers, was also observed.

Philip Landrigan, chairman of community and environmental medicine at Mt. Sinai School of Medicine in New York, updated a 1990 critical assessment of these studies for presentation to the 1993 International Symposium on Health Hazards of Butadiene and Styrene in Helsinki, Finland. All of the epidemiologic studies, Landrigan points out, share several shortcomings. Inadequate assessment of past exposure is the greatest weakness in all these studies, he says. The absence of precise individual data tends to diminish the likelihood of observing an etiologic association. Another shortcoming is the inherent inability of mortality studies to account for the increasing longevity of patients with lymphatic and hematopoietic malignancy that has been achieved with modern chemotherapy. Mortality studies do not reflect incidence. Failure to specifically examine black workers in most of the studies overlooks an opportunity to examine a subpopulation possibly at high risk. There is also a failure to account adequately in cohort studies for the healthy worker effect, resulting in underestimation of excess mortality attributable to butadiene exposure. Finally, comparison to either the general U.S. population or the general population of a particular region may not be as appropriate as comparison to a "blue collar" population. Use of such a group would offer more precise comparison and lead to more accurate estimation of excess mortality.

Evidence that the excess cancer mortality is dose related is supported by observations that mortalities are greatest in production and maintenance workers, but not in office staff. "Typically, production and maintenance workers are the groups most heavily exposed to potentially toxic substances," Landrigan says. He cites as further evidence for a positive dose–response link the observation of greatest excess mortality among workers exposed during the war years, presumably a period when butadiene exposures were especially intense due perhaps to wartime production pressures and possible mishaps during start-up of the industry.

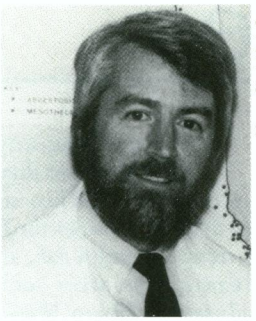
A retrospective study of workers employed at two synthetic rubber plants in Texas found excess mortality for lymphatic and hematopoietic cancer in the older facility. Standardized mortality ratios for lymphosarcoma were two times the normal rate and for leukemia almost three times normal, with excesses for these malignancies most significant among wartime workers.

What may account for the greater leukemia rate among blacks, Landrigan says, is the historical practice of assigning the most menial jobs to black workers. The tasks of predominantly black "labor gangs" included cleaning the inside of tanks that had held butadiene. Racial segregation by job category in at least one Texas facility apparently persisted into the mid-1960s, according to descriptions by long-term workers.

Genevieve Matanoski of Johns Hopkins University has conducted the largest cohort study and most recent case–control studies on butadiene occupational exposure. She also found excess mortality from lymphatic and hematopoietic cancers despite an overall deficit in cancer mortality, with the excess cancers (five times greater than background) most prevalent in production and black workers.

Matanoski points out that cancer was also observed among maintenance and production workers in Canadian plants that had no black

employees. She finds it interesting that higher lymphopoietic malignancy rates among black production workers (likely the most heavily exposed in the sample population) may establish a link with observations in animal studies of increased lymphoid and hematopoietic cancers induced by high concentrations of butadiene.



Mount Sinai Med. Ctr.

Philip Landrigan—Every attempt should be made to prevent exposure to butadiene.

One criticism of the findings in black production workers arose from an analysis that showed lymphopoietic cancers concentrated in short-term workers. John Acquavella of Monsanto's epidemiology department and chair of the International Institute of Synthetic Rubber Producer's epidemiology committee, says excess cancers in short-term workers but not in long-term workers is controversial because of the long latency period required for mortality to occur from these malignancies. "These workers had lymphopoietic cancers when they were first hired or shortly thereafter. We know very little about work histories of people before they entered and after they left the industry, says Acquavella."

"Here Acquavella is under a misconception," Matanoski says. "Average employment of these workers was more than 12 years. That's certainly not short-term." She points out that the cohort study at a butadiene manufacturing facility in Texas showed high risk in short-term workers. "But," she says, "that meant 10 years or less, which is a long employment, and most of the risk is in that group." However, Matanoski agrees there's an absence of data on hiring and employment duration during industry start-up years. "There were new plants, new people without experience moving from one plant to another, and lots of short-term workers became long-term workers."

Several other data classification factors combine to make retrospective cohort analysis difficult for epidemiolgists studying health effects of occupational exposure to butadiene. In spite of attempts to classify jobs by butadiene exposure, job titles are often nonspecific and difficult to classify even in known exposure categories. As Matanoski has reported, one finds compressor operators working in utilities, truckers in operation services, and variation in exposure type by laboratory job. Moreover, jobs within a work area may vary widely in exposure rankings, so that exposed jobs become diluted with nonexposed groups, and thus exposure risk disappears. According to Matanoski, case–control analysis (using continuous variables in a logistic regression model) offers a more specific way of classifying each individual by exposure rank.

Questions have also been raised about interpretation of the epidemiology results because of an apparent lack of consistency in the types of lymphatic cancers observed to be elevated in the different studies. The argument is that such inconsistencies represent etiologically distinct cancers. Landrigan points out, however, that diagnostic categories of lymphatic cancers are imprecise and overlap. Transitions between different lymphatic cancers are frequently seen. In addition, these transitions are complicated by historical changes in nomenclature; certain lymphomas and certain leukemias such as chronic lymphocytic leukemia are considered to represent different clinical expressions of the same process of malignancy.

Says Landrigan, "Based on a very extensive review of the epidemiologic and toxicity literature, butadiene is a serious carcinogen. It has been shown to be able to cause cancers in animals and humans, and the types of cancers commonly result in leukemia and lymphosarcoma. Every attempt should be made to prevent occupational and environmental exposure to butadiene."

Biomarkers and Butadiene Exposure

Although occupational exposures to butadiene have been reduced during recent years, it may not be possible to determine through epidemiologic methods whether current or even proposed levels are adequate to protect workers. This makes biological monitoring techniques very important. Of two urinary metabolites—the product of epoxybutene hydrolysis followed by glutathione conjugation, and the product of glutathione conjugation of epoxybutene—only the former appears to be an effective biomarker of human exposure to butadiene. The product of epoxybutene hydrolysis has been detected in humans exposed to butadiene at concentrations as low as 3–4 ppm.

Johns Hopkins. U.

Genevieve Matanoski—Racial segregation by job category led to higher butadiene exposures for blacks.

Researchers at the University of Texas Medical Branch in Galveston report pilot study findings that indicate the biological significance of exposure to genotoxic chemicals can be evaluated shortly after exposure by measuring the levels of genetic damage in exposed populations. Environmental toxicologist Jonathan Ward, Jr. and his colleagues have investigated the frequency of mutations at the *HPRT* locus in lymphocytes from workers exposed to butadiene. High exposures were approximately 3.5 ppm and low exposures were approximately 0.03 ppm. Workers in the exposed areas had significantly higher frequencies of mutant lymphocytes than both the less exposed and nonexposed subjects. Urine specimens were also collected and evaluated for the presence of butadiene-specific metabolites. Urinary metabolite concentrations also correlated highly with exposure. These results indicate that a biomarker of low-level butadiene exposure in humans is associated with gene mutation. "It is now well established that mutagenesis in cancer genes is a major aspect of carcinogenesis," Ward says. "Therefore, conceptually, if you see an elevation in a population exposed to a particular substance, you should be concerned that the increase would lead to an increase in cancer."

Samples from follow-up studies are just now beginning to be analyzed, Ward says. He points out that current results indicate that an occupational exposure limit proposal of 2 ppm may not be low enough to protect workers from the adverse health effects of butadiene.

Louis Beliczky, former director of industrial hygiene, safety and environmental affairs for the United Rubber Workers, and now a private consultant to industry and trade unions he says that court litigation in Texas and Louisiana on the health effects of occupational exposure to butadiene is beginning to grow. Beliczky would like to see government activity aimed at lowering workplace exposure levels to below 0.5 ppm. "I see no reason why industry can't meet that. I know for a fact that most monomer and polymer facilities can get down to 0.5 ppm," said Beliczky.

Following a review of all the reports and debate, leaders of the Helsinki symposium concluded that occupational exposure to butadiene was "found to be strongly associated with carcinogenic risk." They point to a number of areas still requiring further research to better evaluate effects at various exposure levels. Among these areas are studies on reproductive toxicity and a need for further refinement of physiologically based pharmacokinetic dose–response models to clarify the relationship between exposure, the dose that reaches the target, and biological effects, and an understanding of basic biological mechanisms responsible for the effects observed. According to a number of scientists and health care clinicians, the pursuit of these research objectives should not delay the reduction of human exposure to butadiene.

Leslie Lang

Leslie Lang has previously written for *EHP* about pesticides.